



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:

Dan-Hui Dorothy Yang *et al.*

Serial No.: 10/698,225

Filed: October 31, 2003

For: **Protein Bioarray on Silane-Modified Substrate Surface**

Confirmation No.: 1504

Group Art Unit: 1641

Examiner: Lum, Leon Yun Bon

Docket No. 10021166-1 (50113-1360)

DECLARATION OF MAGDALENA A. BYNUM PURSUANT TO 37 C.F.R. §1.132

Commissioner of Patents
Washington, D.C. 20231

Sir,

I, **Magdalena A. Bynum**, hereby declare that:

- 1) The invention embodied in the above-identified patent application is directed to protein bioarrays on silane-modified substrate surface and methods of producing the protein bioarrays.
- 2) Along with Dan-Hui Dorothy Yang, I am a co-inventor of the above-identified patent application.
- 3) I am advised that the United States Patent and Trademark Office (USPTO) has rejected one or more independent claims presently pending in the above-identified patent application based, at least in part, upon *Butler et al.* (U.S. Patent No. 6,589,726, "*Butler*") in view of *Lefkowitz et al.* (U.S. Patent No. 6,258,454, "*Lefkowitz*"). I am further advised that one or more dependent claims presently pending in the above-identified patent application has been rejected, at least in part, upon *Butler et al.* in view of *Lefkowitz et al.* and further in view of *Haab et al.* ("Protein microarrays for highly parallel detection and quantitation of specific proteins and antibodies in complex solutions", *Genome Biology* 22 January 2001, 2(2), 1-13, "*Haab*"). I have reviewed both the pending Office Action from the USPTO and the cited references.

4) The *Butler* reference generally discloses a method of fabricating a solid support array with patterned hydrophilic and hydrophobic sites. The hydrophilic site is spatially segregated from neighboring hydrophilic sites because of the hydrophobic sites between the hydrophilic sites. A hydrophobic site of *Butler* is typically inert to conditions of in situ synthesis. Solutions of reactants may be added to the hydrophilic sites on the surface, which may support covalent or non-covalent attachment to chemical or biological entities.

5) The *Lefkowitz* reference generally discloses a method of preparing hydrophobic self-assembled surface with different functionality by adjusting the ratio of two silane groups on the surface. In addition, the *Lefkowitz* reference discloses that the functionalized surface prepared using its process has functional groups that enable covalent binding of molecular moieties. See *Lefkowitz* at col. 6, lines 29-31. The *Lefkowitz* reference does not suggest non-covalently attaching a molecule to its surface, or even attaching a protein probe by any type of means.

6) As one of at least ordinary skill in the art, I would not be motivated to combine the *Butler* and *Lefkowitz* references to arrive at the protein bioarrays and methods of producing a protein bioarray claims of the present application. Specifically, neither the *Butler* reference nor the *Lefkowitz* reference discloses a hydrophobic surface modification layer. In addition, neither the *Butler* reference nor the *Lefkowitz* reference discloses a probe protein non-covalently attached via hydrophobic-hydrophobic interactions.

7) Along with my co-inventor, Dorothy Yang, I have discovered that the protein bioarray disclosed in the claims of the instant invention is surprisingly effective as a surface for protein deposition.

8) Namely, we have discovered that the hydrophobic nature of the surface modification layer of the protein bioarray of the instant claims binds protein by strong hydrophobic-hydrophobic interactions.

9) We have also determined that the functionality on the hydrophobic surface modification layer of the protein bioarray of the instant claims can be used to adjust the

surface energy and to provide hydrogen-bonding sites, which in turn increases the van der Waal interaction of proteins with modified surface.

10) In addition, the non-specific binding properties of the proteins on the hydrophobic surface modification layer of the protein bioarray of the instant claims provide another advantage in that the unspotted area can be sufficiently blocked by blocking proteins. This decreases the background caused by labeled target proteins that non-specifically bind to chemically-modified surfaces.

11) To support and/or demonstrate the surprising benefits of the protein bioarray disclosed in the instant claims, we conducted a number of experiments in 2003 prior to filing of the instant patent application.

12) Attached as Exhibit "A" is a portion of a PowerPoint® presentation that we prepared in July 2003 that demonstrates certain results of our experiments with respect to an exemplary protein bioarray covered by the instant claims 1, 11, and 20.

13) On page 2 of the Exhibit a surface comparison for various surfaces generated by an anti-haptoglobulin antibody probe binding to a haptoglobulin target in whole serum. As can be seen, the spots for an undecenyltrichlorosilane (referred to as "UDS" in Exhibit A) are much brighter than those on either a SuperEpoxy surface (produced by TeleChem International, Inc. of Sunnyvale, California) or poly-L-lysine surfaces (referred to as "PLL" in Exhibit A).

14) In addition, as demonstrated in on page 3 of Exhibit A, the background signal for various protein array surfaces were measured by buffer probes. As can be see from the bar chart, the background signal for UDS surface was much lower than PLL surface, and also lower than the Telechem SuperEpoxy surface.

15) Attached as Exhibit "B" is a portion of a PowerPoint presentation that we prepared in 2003 that demonstrates certain results of our experiments with respect to an exemplary protein

bioarray ("UDS"), which is covered by the instant claims 1, 11, and 20. As can be seen from the figures on at least pages 5-6 of Exhibit B, the background signal for UDS surface was much lower than PLL surface.

I hereby declare that all statements made herein are of my own knowledge are true and that all statements are made on information and belief and are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

8/31/2006
Date

Magdalena A. Bynum
Magdalena A. Bynum

Detection of Targets in a Complex Proteins Sample Using T1J Printed Cytokine Abs Array

Antibodies printed:

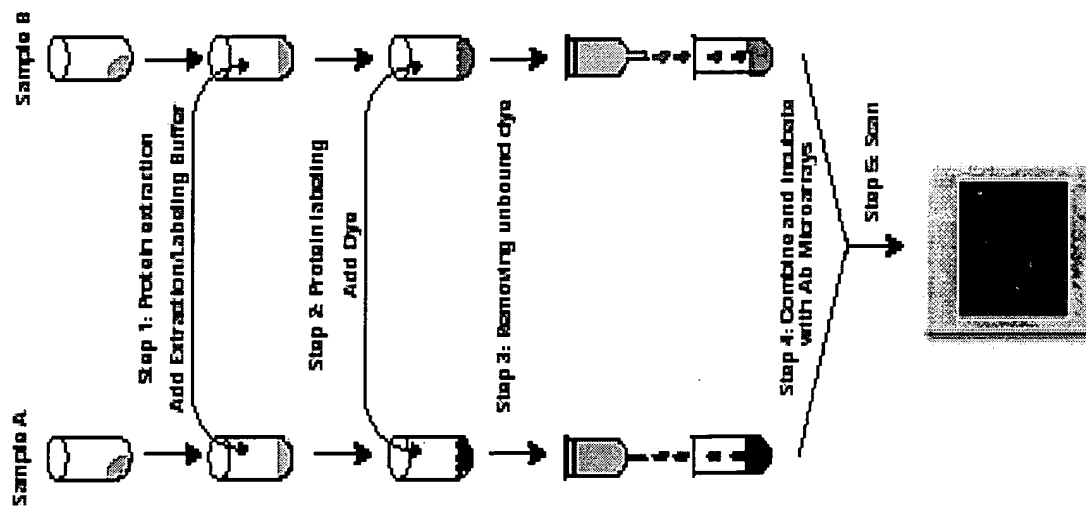
- Rat-Anti-mouse-IgG1
- Anti-TNF-alpha
- Anti-IL-6
- Anti-IFN-gamma

Surface Used:

PLL and UDS

Samples: CAEC, CAEC-OXLDL

Final Labeled Protein
Concentration: ~200ug/ml
(David Deng)

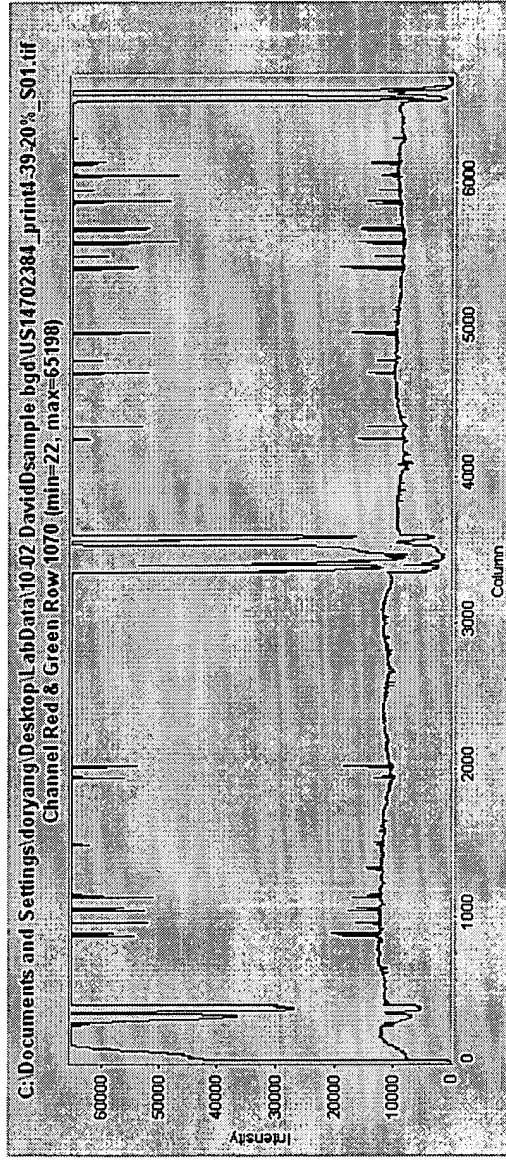
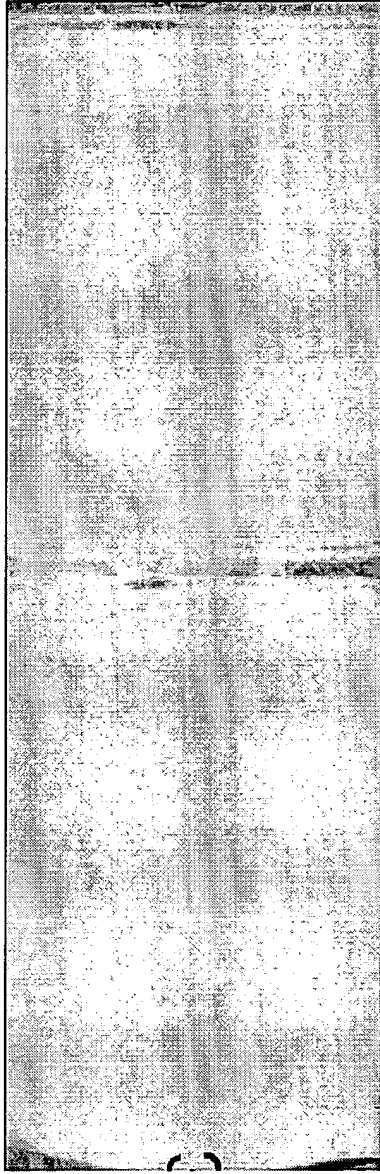


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Experiment:

- 1:20 Dilution for all labeled proteins (10ug/ml each)
- Sample I: Cy3-CAEC + Cy5-CAEC-OXLDL;
Sample II: Cy5-CAEC + Cy3-CAEC-OXLDL
- Casein block from Pierce was used to preblock slide and as binding buffer unless otherwise noted
- Binding step lasted 1.5 hr at r.t. at ~3rpm
- Typical wash

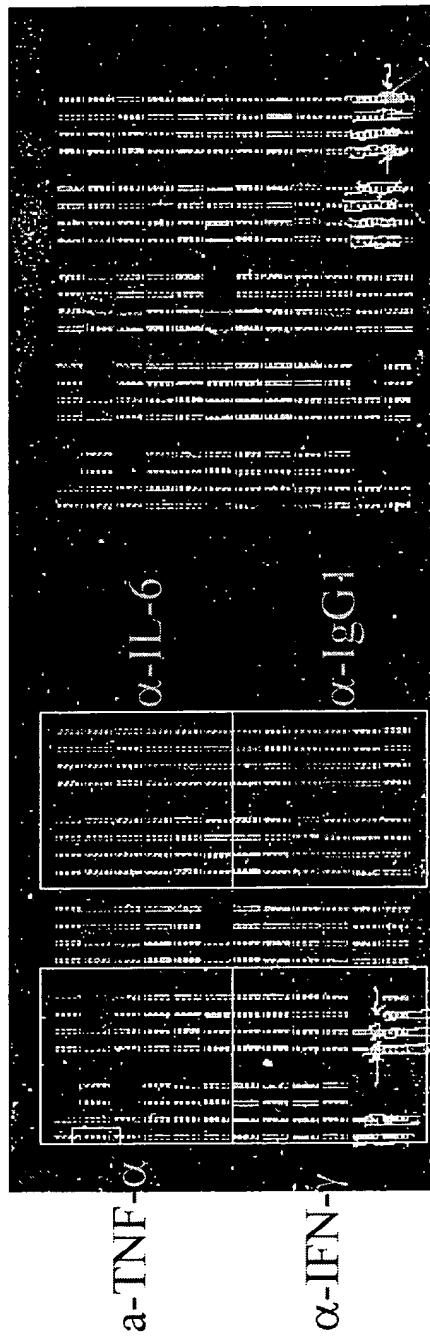
Very High Background on PLL: 20% PMT



6% milk as blocking agent and binding buffer didn't help.

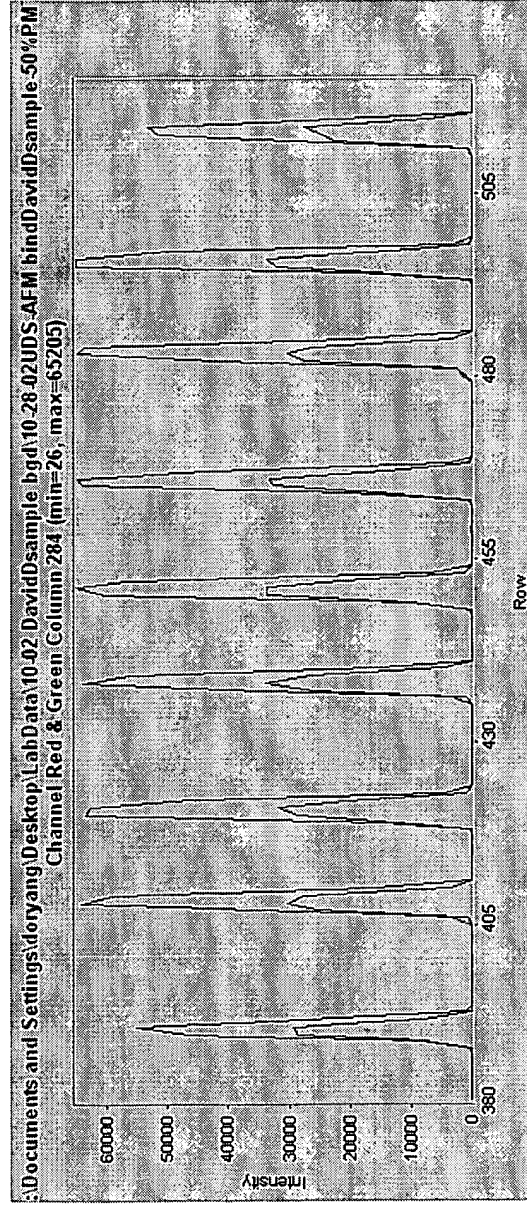
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UDS: 50% PMT (Results repeatable on background)



Left: Sample I
Right: Sample II

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Anti-TNFalpha at 400ug/ml, 2 fires; Cy3-CAEC+Cy5-CAEC-OXLDL